Protein Crystallography Station—Solving Protein Structures with Innovative Time-of-Flight Neutron Diffraction Techniques

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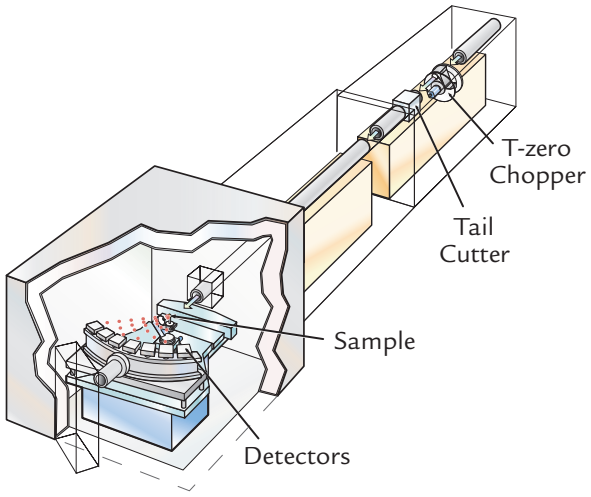
Introduction

Emerging efforts to solve the three-dimensional structures of thousands of proteins sequenced in genome projects are largely based on the use of synchrotron x-rays. However, certain unique types of information relating to structurally and functionally important hydrogen atoms and water molecules can only be obtained through experimentation with neutrons. Hydrogen atoms comprise roughly half the atoms of biological molecules such as proteins, DNA, and carbohydrates, and hydrogen ions are the primary motive force in many fundamental biological processes. Most biological processes take place in an aqueous environment where interaction with water is crucial. Neutron studies in general provide information that cannot be obtained by other methods and are strongly complementary to x-ray, electron microscopy, and nuclear-magnetic-resonance investigations.

A new neutron Protein Crystallography Station (PCS) located at LANSCE incorporates a number of technological innovations that, with the improved LANSCE neutron source and a large electronic neutron detector, will allow the structural biology community to collect time-of-flight (TOF) wavelength-resolved Laue data from small protein crystals and partially disordered systems, including fibers and membranes. The Department of Energy Office of Biological and Environmental Research funds the PCS, which is earmarked for commission in 2001. This innovative station was built by the Biosciences Division with support from LANSCE and is the only resource of its kind in North America. Accommodating fifteen to twenty experiments each year during an eight-month run cycle, this station has the potential to augment the role of a variety of high throughput structural genomics programs with information about the mechanisms of newly discovered enzymes.

Protein Crystallography Station: An Overview

The PCS is located on flight path (FP) 15, viewing a moderator specifically tailored for protein crystallography where neutrons are emitted in pulses at a rate of 20 to 30 Hz.[1] A schematic representation of the beam layout on FP15 is shown in Fig. 1. From the moderator, the neutrons travel 28 m down vacuum pipes with collimation inserts that taper the neutrons to produce a fine, almost parallel, beam that hits the crystal sample. The collimation elements



▲***Fig. 1.*** *PCS beam layout (courtesy of Kathy Lovell and Garth Tietjen).*

extend back into the bulk shielding that surrounds the moderator where a 2-m section of beam pipe can be filled with mercury to act as a shutter for opening and closing the neutron beam.

At 11 m from the moderator, the vacuum pipe carries the neutrons out of the small experimental room (ER1) that surrounds the bulk shield and through a wall into a larger experimental hall (ER2). The vacuum pipe is interrupted in ER1 by two beam-shaping devices at 9.5 m from the moderator—a composite T0/T1 chopper and a proposed tail-cutting device. These devices remove unwanted high- and low-energy neutrons and neutrons that trail too far behind similar energy neutrons in each pulse, thus optimizing the neutron beam for high counting rates and low backgrounds at reasonable instrument resolutions. The vacuum pipe is tightly surrounded by heavy shielding until it reaches the sample position where the shield opens up to a large cave in ER2.

In the cave, neutrons interact with atoms in the crystal sample and are scattered out of the beam in all directions. Neutrons with a wavelength that matches the spacing between a set of periodic planes of atoms are diffracted in a specific direction and produce a spot when recorded by a neutron area detector. Different planes of atoms produce a pattern of diffraction spots—a Laue pattern—that correspond to different wavelengths. A Laue pattern is

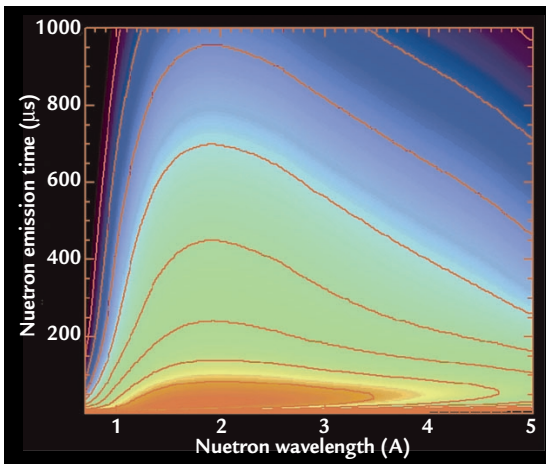
recorded while a κ-circle goniometer moves the crystal and detector between about 30 different orientations. This feature enables all planes in the crystal to be brought into an orientation that will produce diffraction spots. A complete data set can consist of many thousands of diffraction spots. An optional 8-T magnet can be mounted on the goniometer to magnetically orient membrane and fiber samples. A large cylindrical detector collects as many spots as possible at each crystal orientation without having to reposition the detector.

The positions of detected neutrons are passed from the detector to a VXI-based data-acquisition system where they are stored in memory along with their time of arrival. After a sufficient number of neutrons have been collected at a particular crystal and detector setting, the data and the parameters that describe the instrument setting are written to a data archive in the form of a NeXus file. A commercial software package called d*TREK has been customized to control data collection, display, and processing and ultimately to produce a measured intensity and an index for each diffraction spot. The index is a crystallographic description of the diffracting planes that produced the diffraction spots. The intensities and indices provide the input required by conventional Fourier-based protein-structure-refinement software that ultimately produces an image of the protein crystal's structure.

Toward Technological Innovations in Protein Crystallography

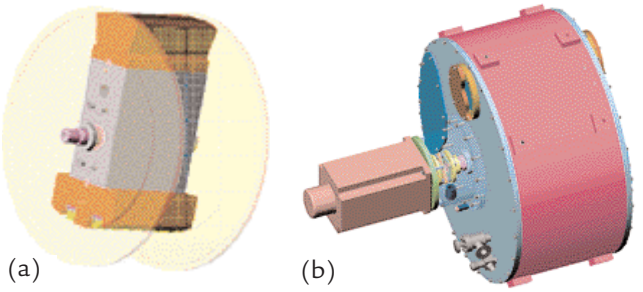
The development of neutron protein crystallography has mainly been aimed at developing strategies that overcome limitations in neutron flux by maximizing the quantity and quality of data. The effort to build a PCS at LANSCE with greatly reduced sample-size requirements has involved a number of new technological innovations. Some of these innovations described below are expected to have general applications outside of structural biology.

A Partially Coupled Water Moderator. High-energy neutrons produced by the target are slowed down to thermal or cold energies by ambient water (~ 300 K) or liquid hydrogen (~ 20 K) moderators that have been neutronically isolated (decoupled) from their surrounding. Decoupling maintains a small neutron pulse width after moderation so that very high energy and diffraction resolutions can be accessed. However, the most useful neutron pulses for structural biology have energies between 3 to 80 meV and do not require short time widths. By partially coupling a water moderator to its surroundings, the neutron flux in the intermediate "cool" energy range (~ 100 K) has been increased at the expense of resolution (Fig. 2).[2] The exact coupling has been optimized along with the instrument FP length (28 m) so that length scales down to 1.5 Å are still resolved.



▲***Fig. 2.*** *Neutron emission time-wavelength probability distribution as calculated by a Monte Carlo neutron and photon transport code for the PCS water moderator located on FP15. The contours are equally spaced on a logarithmic scale. The long-time exponential decay of the pulse becomes clearly visible as the contours become equally spaced (courtesy of Luke Daemen).*

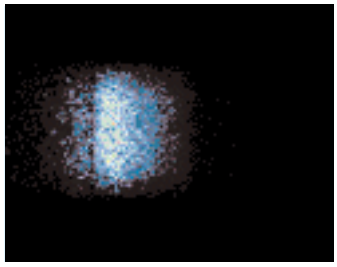
Composite T0/T1 Chopper. The beam is tailored to a wavelength range of 1 Å to 5 Å by a novel composite rotating-blade T0/T1 chopper that is located at 9.5 m from the moderator. The T0 component reduces the amount of background and protects biological samples from damage by removing fast neutrons and γ-rays from the beam. The T1 component removes frame overlap—low-energy neutrons that fall behind one pulse (trailing neutrons) and then interfere with the high-energy neutrons from a subsequent pulse. The T0 component is a rotating nickel alloy blade (Fig. 3a) with a cross section similar to the size of the neutron beam, a length in the beam of around 12 cm, and a radius of 30 cm. The blade rotates at the same frequency as the neutron source and is in a position to block fast neutrons and γ-rays each time that a pulse of protons hits the spallation target. An aerodynamic shroud (opaque in Fig. 3a) coupled to the blade increases the lifetime of the bearings by allowing the chopper housing (red in Fig. 3b) to be filled with helium rather than a vacuum. A neutron-absorbing paint on one end of the shroud makes a T1 blade with a window large enough to transmit 1-Å to 5-Å



▲***Fig. 3.*** *(a) The chopper blade and aerodynamic shroud, and (b) the chopper housing, as described in the text (courtesy of Gerry Haas, Joe O'Toole, and Mark Taylor).*

neutrons. T0 and T1 choppers are usually installed on separate motors; however, a composite chopper greatly reduces cost and maintenance.

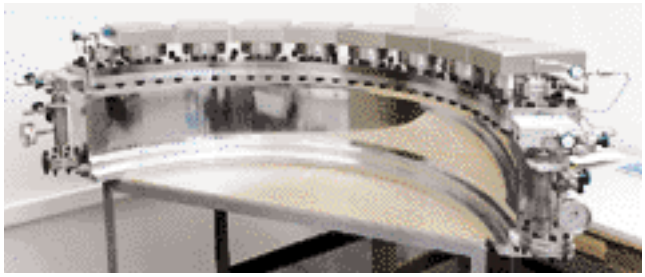
Tail-Cutting Device for High Resolution. Most protein crystals diffract to resolutions that correspond to length scales of ~ 2 Å. In its standard configuration, the PCS can access length scales down to ~ 1.5 Å. However, for situations where unusually high diffraction resolution is required (< 1.5 Å) or when trailing neutrons cause diffraction spots to overlap, a novel tail-cutting device has been designed.[3] A nested aperiodic multilayer stack moves over an angular range of about 1.5° at the same frequency as the beam. This stack diffracts unwanted trailing neutrons in the long time tail of the neutron pulse out of the transmitted beam (Fig. 4). This device is a relatively cheap alternative to a Fermi chopper and has the advantage that it is easy to maintain.



▲***Fig. 4.*** *Beam spot of 2-Å neutrons Bragg-reflected by the tail-cutting device out of the main transmitted beam. The "useful" transmitted neutron beam spot is centered on x = 0, y = 0, which is the beam spot at a distance of 5 m from the tail-cutting device. At a distance of 10 m from the tail-cutting device, the tail neutrons are sufficiently (spatially) separated from the early neutrons so that they can easily be removed with a small beam stop.*

A Cylindrical Neutron Detector. In addition to maximizing the number of useful neutrons that hit the crystal sample, data-collection efficiency can be greatly increased by maximizing the number of diffracted neutrons that are collected by the detector. The PCS incorporates an advanced-design gas, multiwire, proportional detector chamber. The detector, built by the Instrument Division of Brookhaven National Laboratory (Fig. 5), has a height of 20 cm and a curved horizontal dimension that subtends 120° at the sample position. The detector's 70-cm radius takes into consideration the peak-to-background ratio, spot overlap, the detector spatial resolution, and engineering considerations. Parallax and window thickness limits the height of the detector. Although neutron-image-plate detectors and scintillation detectors are cheaper, can be made larger, and have higher absolute counting rates, they have other features that limit their usefulness for TOF protein crystallography.

The PCS detector has a novel electrode arrangement and signal-processing system that gives a contiguous detecting medium of 150 cm x 20 cm with a spatial resolution of 1.2 mm and a maximum counting rate of 10^6 ns^{-1}.



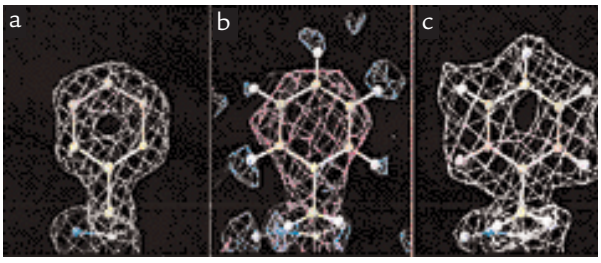
▲***Fig. 5.*** *The PCS cylindrical neutron detector. The detector consists of an electrode structure contained in an aluminum pressure vessel. The pressure vessel is filled with a mixture of ^3He and propane. The ^3He has an extremely high cross section for thermal neutron absorption. Neutrons diffracted by the sample are absorbed by the ^3He. This interaction results in the creation of a proton and triton. These primary ionization products drift toward the nearest electrode-anode wire where they multiply in the high electric field near the wire surface. The charges induced over different electrodes allow for the spatial detection of an event.*

Shielding. Shielding at a spallation neutron source is more difficult than at a reactor because of the presence of high-energy neutrons. As part of the Accelerator Production of Tritium project at Los Alamos, selected neutron cross sections have been extended up to 150 MeV, and Monte Carlo code has been developed to use these cross-section libraries. LANSCE has developed a technique for using Monte Carlo codes to calculate absolute neutron and γ-ray dose rates at the surface of neutron-beam-line shields. This technique has been applied to the design of the PCS shielding.[4] A variety of laminated shields of iron/polyethylene/iron, borated wax, and magnetite concrete around different beam-line components (e.g., choppers, scrapers, experimental caves, and beam stops were considered) have been investigated with a view to maximizing the efficiency of the shielding and minimizing the material costs. The section of vacuum pipe in ER1 is shielded by an iron and polyethylene laminate, with some of the polyethylene impregnated with boron. In ER2, a magnetite concrete and polyethylene shield is used. The sample cave is constructed from monolithic blocks bolted together. Each block corresponds to a steel container filled with 8 in. of 5% borated polyethylene. The interior walls of the cave are also lined with 1 in. of polyethylene.

Research Applications

Hydrogen atoms participate in interactions that determine the structure and properties of biological macromolecules and materials. (Macromolecules are composed of much larger numbers of atoms than ordinary molecules. Biologically important proteins and nucleic acids are among the many substances that are made up of macromolecular units.) But determining hydrogen positions in crystals, fibers, or membranes of biological macromolecules is often difficult. Because of their low scattering power for x-rays, hydrogen atoms are generally not seen in protein or biological polymer structures determined

by x-ray crystallography except at ultra-high resolutions (< 1 Å). In the Protein Data Bank,[5] the sole international repository for macromolecular structure data, fewer than 1% of structures attain these resolutions—the majority of structures fall well below (> 2 Å). The scattering of neutrons by hydrogen or deuterium is comparable to that of other atoms with the result that neutron diffraction has been used to obtain the positions of hydrogen atoms at typical diffraction resolutions (Fig. 6). In studies of enzyme mechanisms, neutron protein crystallography has been a powerful tool for determining key details of hydrogen structure around catalytic sites. Much of our detailed understanding of serine-proteinases, aspartic proteinases, and glucosidases has come from neutron diffraction. The unique information that can be derived from neutron protein crystallography is of exceptional importance in the understanding of enzymatic mechanisms. Similarly, the protonation states of functionally important residues in the oxygen-transport protein, myoglobin, have long been of interest, and neutron diffraction has provided direct evidence of these states.



▲***Fig. 6.*** *Details of scattering density maps calculated from x-ray and neutron data collected from myoglobin, a protein found in muscle cells that functions as an oxygen-storage unit, providing oxygen to the working muscles. Fig. 6a shows the scattering density of myoglobin calculated from 1.8-Å x-ray data. Only carbon atoms are covered. Fig. 6b shows the negative (blue) and positive (red) scattering density of myoglobin calculated from 2-Å neutron data. Hydrogen with its strong negative scattering length is covered. Fig. 6c shows the positive scattering density calculated from 2.2-Å neutron data collected from perdeuterated myoglobin. Deuterium with its strong positive scattering length is covered. Neutron diffraction has been used to locate individual protons at catalytic sites.*

Much of the activity of biological macromolecules takes place in water. Changes in the aqueous environment can dramatically change the structure and function of biological macromolecules. At the resolution of most x-ray diffraction studies, water molecules are located with very poor reliability. Because D_2O molecules diffract neutrons strongly, ordered water molecules can be detected as easily in a neutron study as a sulfate ion in an x-ray study. Neutron diffraction studies on the vitamin B_{12} coenzyme, DNA, and other proteins and hormones have provided a wealth of unique information on the hydration of biological macromolecules.

Conclusion

Neutron diffraction has provided many important contributions to our understanding of biological structure, function, and dynamics over the past thirty years. Today the study of biological structure is going through a transformation. An explosion of three-dimensional protein structures is expected from structural genomics. The structure of complex systems consisting of assemblies of smaller proteins and nucleic acids are being studied to understand cellular processes. Biomaterials, biosensors, and food technology are becoming more important. Neutron diffraction can play an important role by providing unique types of information in this rapidly expanding field. As the only facility of its kind in North America and as a worldwide state-of-the-art instrument, the PCS will be one of the main tools for realizing this potential.

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